secretion of antibodies, and those that do produce antibodies can be separated from those that do not produce antibodies, if desired.

**[0134]** As discussed herein, B cells that produce antibodies that bind to or otherwise favorably interact with the agent of interest (and the droplets that contain these B cells) can be identified and/or separated from B cells that do not produce these particular antibodies. This process may involve the use of one or more signaling entities, as described herein.

**[0135]** For B cells that produce a first antibody which associates with all or a portion of an agent of interest, the nucleic acid encoding for the production of the first antibody may be extracted. For example, the sequence of that cell's antibody heavy (VH) and/or light (VL) chains can be extracted. In some embodiments, this extraction is performed by rupturing the cell without breaking the droplet. In some cases, however, the droplet can be broken during the extraction process.

**[0136]** The DNA from the cell may be sequenced using any suitable technique known to those of ordinary skill in the art. Examples of DNA sequencing techniques include, but are not limited to, PCR (polymerase chain reaction), "sequencing by synthesis" techniques (e.g., using DNA synthesis by DNA polymerase to identify the bases present in the complementary DNA molecule), "sequencing by ligation" (e.g., using DNA ligases), "sequencing by hybridization" (using DNA microarrays), nanopore sequencing techniques, or the like. Optionally, the extracted nucleic acid sequence may be amplified, duplicated, or expanded by PCR, rolling circle replication or equivalent techniques.

**[0137]** In one set of embodiments, the droplets are used in combination with PCR. For example, in some cases a normal PCR mixture is divided between the aqueous droplets of a water/oil emulsion such that there is, in most cases, not more than one template DNA molecule per droplet. The emulsion then may be thermo-cycled and each of the template DNA molecules may be amplified in a separate droplet. However, in other embodiments, the droplets are first broken, then the nucleic acid sequenced using PCR or other sequencing techniques known to those of ordinary skill in the art.

**[0138]** The extracted (or duplicated) nucleic acid sequence may be inserted into a host cell (e.g., an immortalized cell such as a CHO cell, etc.) that can subsequently express the antibody. This cell can then be used to produce a second antibody, and the cell may be optionally cloned or otherwise cultured for further antibody production. Examples of methods of transfecting a cell with a nucleotide sequence are well-known to those of ordinary skill in the art, and are described in greater detail below.

**[0139]** However, it should be understood that in some cases, no host cell is needed. For instance, the antibody or other species may be produced in a cell or in a cell-free expression system. Cell-free translation systems will often comprise a cell extract, typically from bacteria (Zubay, G. (1973) Annu. Rev. Genet., 7, 267-287; Zubay, G. Methods Enzymol., 65, 856-877; Lesley, S. A. (1991) J. Biol. Chem. 266, 2632-2638; Lesley, S. A. et al. (1995) Methods Mol. Biol. 37, 265-278), rabbit reticulocye (Pelham and Jackson, (1976), Eur. J. Biochem, 67, 247-256), wheat germ (Anderson, C. W. et al. (1983) Methods Enzymol, 101, 635-644), etc., or are partially recombinant, cell-free, protein-synthesis systems reconstituted from elements of systems such as the *Escherichia coli* translation system (Shimizu, Y. et al. (2001) Nat. Biotechnol. 19, 751-755). Commercial cell-free translation systems are available from a number of suppliers including Invitrogen, Roche, Novagen, or Promega.

**[0140]** In some cases, the first antibody produced by the B cell is the same as the second antibody produced by the antibody-producing cell, since the nucleic acid inserted into the antibody-producing cell encodes for the production of the first antibody. However, in some instances, misfolding or other events (e.g., different types of posttranslational modifications) can occur during antibody production. In some cases, such differences may arise from different cell types, and/or different cell species. This may result in the formation of, for example, a second antibody that has a different structure than the first antibody, but has the same activity as the first antibody. Alternatively, a second antibody that has a different structure and different activity than the first antibody may be produced.

**[0141]** In order to verify the binding and/or activity of the second antibody, a second antibody or antibody-producing cell that produces a "hit" may be tested as described herein and/or by conventional tests. Furthermore, in some cases, the second antibody may be further optimized, e.g., by directed evolution, and/or further screened to produce an antibody (e.g., a third antibody) having more optimal activity or binding.

**[0142]** As an example of directed evolution techniques, a nucleotide sequence encoding an antibody or a fragment of an antibody may be subjected to various mutation, expressed in cells, then the antibodies having desired characteristics or features (e.g., determined using assays as discussed herein) selected (for instance, using techniques such as those discussed herein, or other techniques) and subjected to further mutations. Mutations can be introduced by a variety of techniques in vivo, for instance, using mutator strains of bacteria such as *E. coli* mutD5, or using the antibody hypermutation system of B-lymphocytes. Random mutations can also be introduced both in vivo and in vitro by chemical mutagens, or ionising or UV irradiation, or incorporation of mutagenic base analogs. Random mutations can also be introduced into genes in vitro during polymerization for example by using error-prone polymerases. Further diversification can be introduced by using homologous recombination either in vivo or in vitro.

**[0143]** The second (or third) antibody or a derivative thereof may also be administered, in some embodiments, to a subject in a therapeutic amount (e.g., "passive immunization"). This may allow, for instance, an amplification of an immune response of the subject from where the original sample was taken, and/or conveyance of some of the immune response of the subject who provided the sample to other subjects. In some embodiments, the second (or third) antibody or a derivative thereof can be used in combination with other therapies or used to direct reagents to work against the original "agent"; it may also be used, in some cases as a diagnostic reagent when included in a measurement system that can assay antibody binding or activity against a sample.

**[0144]** In administering the antibodies to a subject, dosing amounts, dosing schedules, routes of administration, and the like may be selected so as to affect known activities of these compositions. Dosages may be estimated based on the results of experimental models, optionally in combination with the results of assays of compositions of the present invention. Dosage may be adjusted appropriately to achieve desired drug levels, local or systemic, depending upon the mode of administration. The doses may be given in one or several